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Impact of land management and fertiliser use on soil microbial function

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A Dissertation

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C. A. Horne

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Abstract of a Dissertation submitted in partial fulfilment of the requirements for the Degree of Bachelor of Agricultural Science with Honours.

Impact of land management and fertiliser use on soil microbial function

by

C. A. Horne

Soil microorganisms are a key component for soil health and fertility as they promote the turnover of organic matter and nutrient cycling (e.g. phosphorus and nitrogen), which in turn determines plant and animal production. Assessment of soil microbial function can be achieved via various methods based on physiological processes, of which substrate-induced respiration is thought to be the most sensitive to changes in the microbial community. Therefore, MicroResp™, a method of substrate-induced respiration, was used to assess microbial function for this study. Samples were taken from a long-term fertiliser (superphosphate) trial at Winchmore and theLong-Term Ecology Trial (LTET, biomass retetion or removal) at Lincoln University, together with a short-term dairy shed effluent trial (DSE applications) carried out at LTET. The results of this experiment showed season had a significant impact on soil microbial function, primarily due to changes in soil temperature, and to a lesser extent, soil moisture. Treatment effects of the long-term application of superphosphate and the DSE applications on microbial function were not signifcant, however, the effect of biomass retention or removal on the LTET were significant. This is primarily due to changes in soil organic carbon levels and pH which occurred under the contrasting treatments of the LTET, but not for the Winchmore and DSE treatments.

Keywords: Microbial function, MicroResp™, Season, Superphosphate, Dairy shed effluent, Biomass retention, Biomass removal.

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1. Introduction

Soil microorganisms is a general term which encompasses bacteria, fungi and archaea (Coleman, Crossley, & Hendrix, 2004; Killham & Prosser, 2014) and represents over 95% of the soil biomass (Condron et al., 2010). Microbial function is key to overall soil health and fertility due to its role in the turnover of soil organic matter (SOM) as well as key elements (Standing & Killham, 2012; Bardgett, 2016). These functions can make nutrients available for plant production, but also transform nutrients into forms easily lost from the soil (Hopkins & Dungait, 2010). Furthermore, the relationship between microbes and plants tend to be mutualistic, since bacteria and fungi congregate in the rhizosphere where they can utilise sugars exuded from plant roots (Balser et al., 2010). Therefore, changes in land use management which affects microbial substrate utilisation will affect microbial function.

The long-term application of mineral fertilisers, such as superphosphate, does not have significant effects on microbes directly (Bünemann, Schwenke, & Van Zwieten, 2006). However, Bünemann et al. (2006) reported that microbial function has been shown to increase primarily due to an increase in plant production and SOM levels. Furthermore, acidification of soil occurs after long-term fertiliser application which will also decrease microbial biomass, and consequently microbial function, unless lime is applied to counter this (Tilston et al., 2010). Additionally, Tilston et al. (2010) reported the addition of animal manure, such as dairy shed effluent, increases SOM. Subsequently, the soil microbial community increases in size (Anderson & Domsch, 1989), activity (Bolton et al., 1985), and diversity (Hassink et al., 1991). The result is an increase in plant production, and an improvement in both soil structure and soil health (Tilston et al., 2010). In contrast, long term biomass removal reduces organic matter inputs to the soil compared with returning all biomass (Simpson et al. 2012), which has been reported to cause reduced microbial biomass and diversity (Anderson & Domsch, 1989; Adair, Wratten & Lear, 2013).

Season has significant and varied effects on microbial biomass and activity due to seasonal patterns of soil temperature, moisture and plant growth which influence substrate availability (Arnold et al., 1999; Kaiser & Heinemeyer, 1993). Arnold et al. (1999) reported that microbial biomass tends to be greatest in autumn and spring, and lowest in summer and winter. However, Lynch & Panting (1980) found that microbial biomass is three times

greater in spring and summer compared with autumn and winter. Furthermore, Blume et al. (2002) reported that microbial activity in the surface soil is higher in summer than winter. There is a strong relationship between temperature and microbial activity which is most likely due to the diminishing ability of microbes to assimilate organic matter with decreasing temperatures. However, Van Gestel, Ladd, & Amato (1992) reported changes in microbial capabilities are more closely related to seasonal fluctuations in soil moisture rather than temperature.

2. Review of Literature and Research Objectives

2.1. Soil Microbial Function

Microorganisms are key to the formation and degradation (maintenance) of soil organic matter and contribute to overall soil health and therefore, fertility (Standing & Killham, 2012; Bardgett, 2016). Efficient cycling of key elements such as carbon (C), nitrogen (N), and phosphorus (P) is dependent on microbial activity in the soil to degrade organic matter and detritus, and to transform molecules into plant available forms (Hopkins & Dungait, 2010). Soil biota interact with microorganisms in the soil which can either have a positive or negative effect on microbial function (Bottomley, 1998; Morris & Blackwood, 2012).

2.1.1. Microbial Requirements

The energy and nutrients required to grow and sustain the microbial population in soil are obtained from plant and animal detritus along with root exudates (Condron et al., 2010; Morris & Blackwood, 2012). Microbes assimilate the organic carbon together with associated nutrients into carbohydrates, lipids and proteins (Coleman et al., 2004). These products are used by the microbes for metabolism, biomass synthesis and reproduction (Six et al., 2006), however, over 40% of the chemical bond energy released during the process is lost as carbon dioxide (CO_2) and metabolic heat (Coleman et al., 2004). For the most part, microorganisms gain energy through the oxidation of SOM (Whalen & Sampedro, 2010). Carbon is the most important element in terms of microbial activity as it is the primary driver of microbial growth (Hopkins & Gargait, 2010). However, N can also be used by some bacteria as an energy source under specific conditions (Morris & Blackwood, 2012) and ammonia-oxidising archaea use ammonia as their sole energy source (Leininger et al., 2006). Bacteria and archaea are defined as prokaryotes, are both single celled organisms, and are both of similar size, but they are quite different in many fundamental properties (Killham & Prosser, 2014; Alexander, 1998). Although there are distinctions between the two, they are both often referred to as bacteria (Alexander, 1998) as opposed to fungi which are involved with soil organic matter production, decomposition and sequestration (Taylor & Sinsabaugh, 2015). Fungi live in a close symbiotic relationship with plant roots which provide the fungus with soluble carbon in exchange for improved access to soil nutrients (Condron et al., 2010).

2.1.2. Carbon Dynamics

Soil organic matter is the primary carbon substrate providing energy to the soil microbial population (Wagner & Wolfe, 1999; Wollum, 1998). Microorganisms decompose the organic carbon molecules to obtain energy for growth of the microbial biomass with a portion released as CO_2 (Hopkins & Gargait, 2010; Coleman et al., 2004). Figure 1 shows the importance of microbes in the carbon cycle. Bacteria and fungi are attributed to 85-90% of the soil carbon flux as they are the primary driver of decomposition of organic carbon (Hopkins & Gregorich, 2005). The ratio at which carbon is incorporated into the microbial biomass compared to the amount of CO_2 lost is a measure of microbial efficiency (Wagner, 1975). Efficiency can reach up to 65% (Gilmour & Gilmour, 1985), however is dependent on the environment in which the microbes are situated and the material being decomposed (Wagner & Wolfe, 1999). Specific archaea can use CO_2 and H_2, as well as other substrates to form methane, leading to decomposition of organic matter and the release of methane into the atmosphere (Whalen & Sampedro, 2010). Fungi are mediators in organic matter production, decomposition and sequestration (Taylor & Sinsabaugh, 2015). As fungi generally have a greater tolerance of acidity, decomposition of organic matter in acidic soils is for the most part, a fungal process (Killham, 1994).

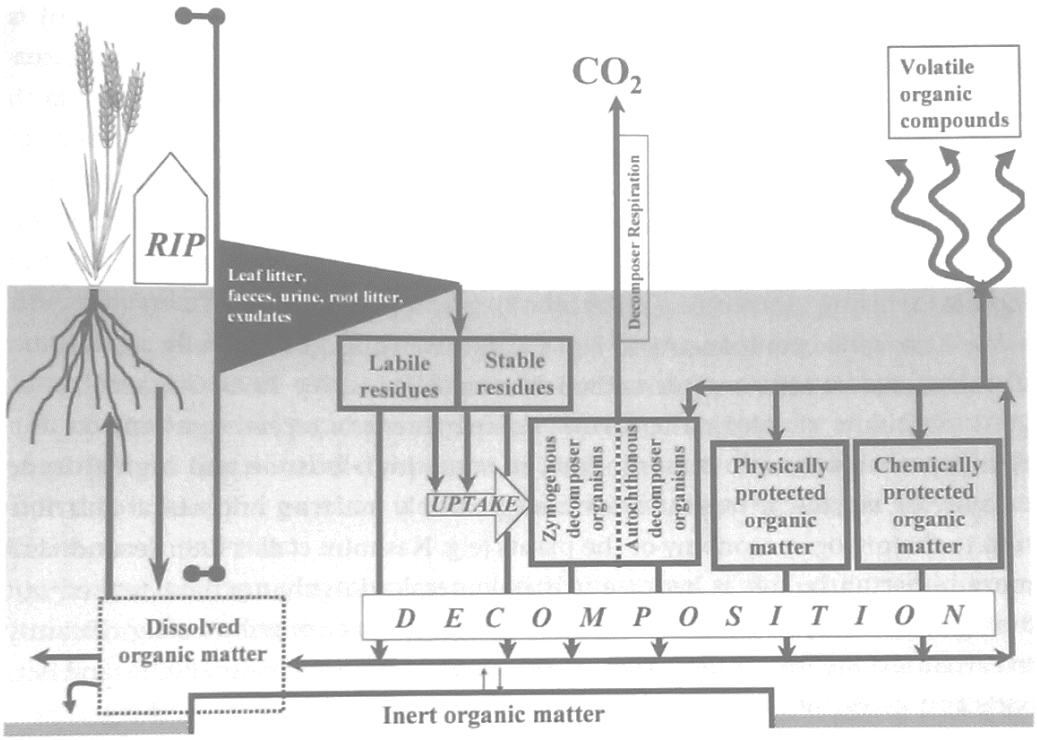

Figure 1. Decomposition and carbon turn-over in soil (Retrieved from Hopkins & Gregorich, 2005).

2.1.3. Nitrogen Dynamics

Microbes play an important role in many aspects of the N cycle including mineralisation, nitrification, denitrification, and fixation of atmospheric N (Hopkins & Gargait, 2010). Fungi are specifically involved in assimilation and dissimilation denitrification (Taylor & Sinsabaugh, 2015). Figure 2 shows the importance of soil microbes in the N cycle.

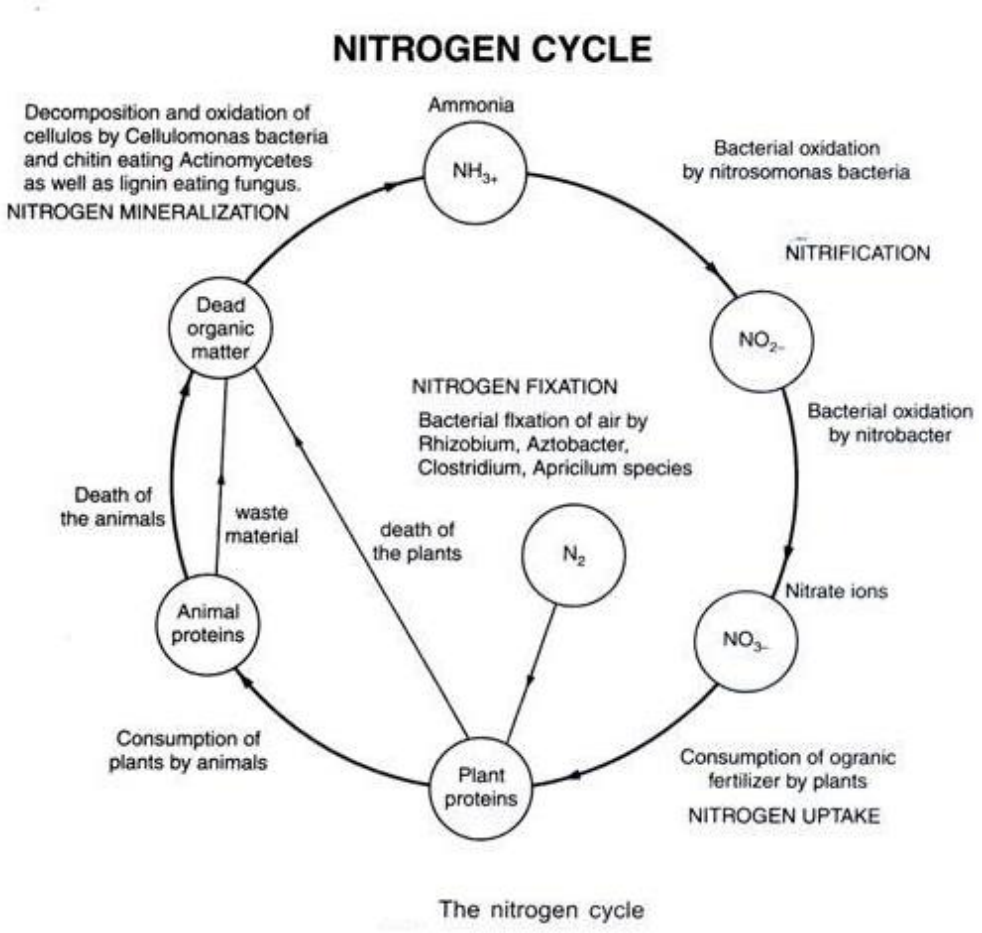

Figure 2. Simple nitrogen cycle (Retrieved from Samiksha, 2015).

2.1.3.1. Mineralisation

Nitrogen mineralisation occurs when organic matter is broken down by microbes to release inorganic forms of N for plant uptake (Haygarth et al., 2013). The inorganic form produced is primarily ammonium, and requires microbial extracellular enzymes to break down the organic – nitrogen polymers (Myrold, 1998). The release of inorganic N by microbes into the soil is dependent on the C to N ratio of the substrate as low N levels in the substrate (C:N > 20:1) can lead to the assimilation of soil inorganic N to microbial tissues (Hopkins & Gargait, 2010). Therefore, when N is limited, the microbial biomass immobilizes N and therefore, reduces plant availability (Haygarth et al., 2013). The relationship between mobilisation and

immobilisation can alter the amount of N in the soil pool, influencing plant availability, denitrification losses and leaching losses (Hopkins & Gargait, 2010).

2.1.3.2. Nitrification

Nitrification is the oxidation of ammonia (NH_3) to form nitrate (NO_3^-) in a two stage process performed for the most part by bacteria (Myrold, 1998; Hopkins & Gargait, 2010). The first stage, ammonia oxidation, is undertaken by ammonia-oxidizing bacteria ('Nitroso-bacteria') which convert NH_3 NO_2^- (Myrold, 1998). However, it has been discovered that specific archaea also have the *amoA* gene that codes for the enzyme responsible for this phase (Di et al., 2009). Although these specific archaea are present in larger numbers in the soil compared with their bacterial counterpart (Leininger et al., 2006), Di et al. (2009) found that nitrification is driven by bacteria rather than archaea, but archaea may catalyse the process (Whalen & Sampedro, 2010). The second stage, nitrite oxidation, is where NO_2^- is further oxidized to form NO_3^- by 'Nitro-bacteria' (Myrold, 1998). The 'Nitro-bacteria', 'Nitroso-bacteria', and ammonia-oxidizing archaea rely on NO_2^- or NH_3 as their sole energy source (Haygarth et al., 2013; Hopkins & Gargait, 2010; Leininger et al., 2006). Therefore, they are reliant on the availability of NO_2^- or NH_3 and will rapidly oxidize any free ions in soil (Hopkins & Gargait, 2010). Nitrification does not inhibit plant uptake of N but can predispose N to denitrification and leaching (Haygarth et al., 2013).

2.1.3.3. Denitrification

Denitrification continues from nitrification as a form of dissimilation (Myrold, 1998), whereby NO_3^- is reduced to gaseous N products (Hopkins & Gargait, 2010). Many different microorganisms carry out this process to form nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2) under anaerobic conditions (Haygarth et al., 2013). This process releases a small amount of energy for microbes to utilise which is important in soils where carbon may be limited or unavailable (Myrold, 1998). Fungi play an important role in denitrification, as it has been shown *Ascomycota* fungus is responsible for a large fraction of the efflux in N_2O (Taylor & Sinsabaugh, 2015). In contrast with dissimilation, assimilation of N occurs when inorganic N is immobilised by microbes or plants (Myrold, 1998). Fungi in symbiosis with plants play a vital role in assimilation as they assimilate N for both production of fungal biomass and to supply the host plant (Taylor & Sinsabaugh, 2015).

2.1.3.4. Nitrogen Fixation

Specialised bacteria have the ability to reduce atmospheric N_2 to NH_3 and incorporate it into amino acids for protein synthesis (Haygarth et al., 2013). This process is carried out by bacteria living in symbiosis with plant roots, either in association with plant roots or free in the soil (Hopkins & Gargait, 2010). The most well-known of these is *Rhizobium* which enters the roots of leguminous plants fixing N_2 in exchange for photosynthetic substrates (Killham, 1994; Whalen & Sampedro, 2010). Actinobacteria *Frankia* can form similar relationships with the roots of non-leguminous plants such as alder (Graham, 1998; Haygarth et al., 2013; Hopkins & Gargait, 2010). Both *Rhizobia* and *Frankia* incorporate large amounts of N into the soil system, increasing soil fertility and decreasing the need for N fertilisation in agricultural systems (Haygarth et al., 2013). Free-living N fixers such as *Azotobacter* and *Azospirillium* live in association with plant roots as they use root exudates in the rhizosphere as an energy source to fix N_2 (Zuberer, 1998; Hopkins & Gargait, 2010). Cyanobacteria is unique in that it can both fix N and perform photosynthesis (Whalen & Sampedro, 2010), allowing it to be independent of organic matter as energy required for N fixation is provided through CO_2 fixation (Killham, 1994). Microbial populations that carry out specialised processes such as N fixation and nitrification are restricted and can become limiting (Paul, 2014).

2.1.4. Phosphorus Dynamics

Another important element in the soil is P, which microbes mineralise and play a role in P dissimilation (Hopkins & Gargait, 2010). Phosphorus is important for energy storage and transfer within the cells (Whalen & Sampedro, 2010). Figure 3 shows the importance of microbes in the P cycle. Mineralisation of P is the process whereby organic P is mobilised by extracellular phosphatase enzymes to inorganic, plant available forms (Haygarth et al., 2013; Mullen, 1998). As with N, the relationship between mobilisation and immobilisation is dependent on the C:P ratio in soil (Whalen & Sampedro, 2010; Mullen, 1998). Where the C:P ratio is greater than 100:1, immobilisation of P occurs by microbes as they have a high P requirement (Haygarth et al., 2013).

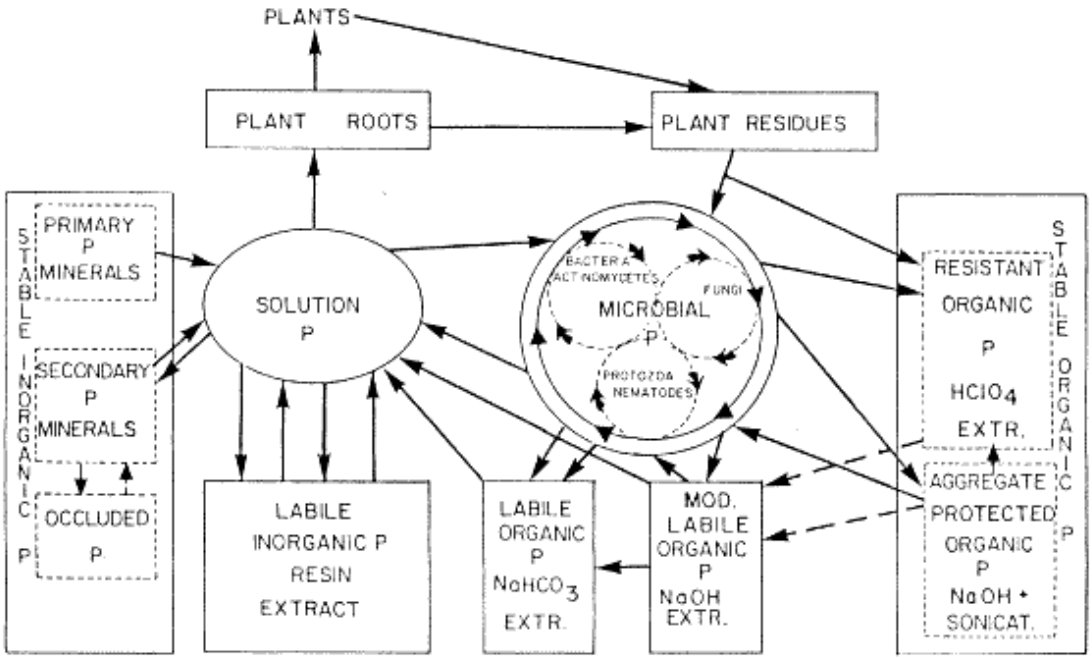

Figure 3. Phosphorus cycle (Retrieved from Stewart, Cole, & Maynard, 1983).

Fungi promote solubilisation and increase the uptake of P from the soil (Hopkins & Gargait, 2010). Both endo- and ectomycorrhizal fungi form a symbiotic relationship with plant roots primarily to acquire photo-assimilated carbon from the plant (Killham, 1994). In return, the mycorrhizal hyphae grow out into the soil and facilitate the uptake of nutrients, especially phosphate ions (Coleman et al., 2004; Allen, 1991). Figure 4 shows the ability of the fungal hyphae to grow past the P depletion zone in order to access more P for itself and the host plant. Solubilisation occurs when inorganic P is released from calcium (Ca), iron (Fe) and aluminium (Al) phosphate compounds, making P available to plants (Mullen, 1998). Fungi enhance solubilisation by releasing organic acids with low molecular weight to increase the weathering rates of these compounds (Haygarth et al., 2013). Increased solubilisation and mineralisation increases P availability for both plants and microbes.

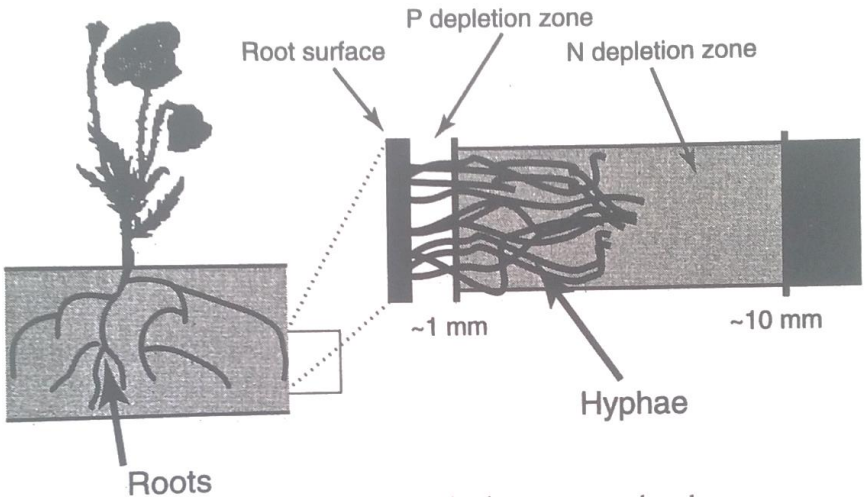

Figure 4. Interaction between fungal hyphae and plant roots which facilitates greater acquisition of soil nutrients (Retrieved from Sylvia, 1998).

2.1.5. Soil Biota Dynamics

Soil biota also includes micro-, meso- and macro-fauna, which interact with soil microorganisms (Condron et al., 2010). Given the constraints of nutritional limitations and environmental stresses placed on soil biota, interactions can be both negative and positive (Bottomley, 1998; Morris & Blackwood, 2012). Negative interactions include predation (a form of exploitation), competition, and amensalism, while positive interactions include commensalism, synergism, and mutualism (or symbiosis) (Bottomley, 1998).

2.1.5.1. Negative Interactions

Predation or exploitation occur when energy or nutrients are transferred from the prey to the consumer (Morris & Blackwood, 2012). For example, microorganisms can be a food source for macro-fauna such as nematodes and protozoa (Condron et al., 2010). Increased population densities of these predatory organisms in the soil are important in releasing microbial nutrients, providing plants with nutrients and ensuring nutrient turnover (Killham, 1994). Competition occurs when two or more different populations or individual organisms both have an active requirement for the same resource (Bottomley, 1998; Killham, 1994). This may limit the number of species and size of microbial populations that the soil is able to support (Morris & Blackwood, 2012). In some cases, amensalism can occur, where a population produces a growth-inhibiting substance in order to gain a competitive advantage (Bottomley, 1998). For example, sulphur (S)-oxidising organisms can acidify the soil which can severely impact acid sensitive species of microorganisms (Germida, 1998).

2.1.5.2. Positive Interactions

Soil fauna can indirectly stimulate microbial populations and activity by increasing the availability of organic matter for decomposition (Condron et al., 2010). This is an example of commensalism, where one population or organism benefits while the other is unaffected (Bottomley, 1998). Mutualistic or symbiotic relationships are relationships where both populations or organisms benefit so that they are able to survive in environments in which they would not be able to survive alone (Morris & Blackwood, 2012; Bottomley, 1998). This is best seen in the symbiotic relationship between mycorrhizal fungi and plant roots, but can occur between soil biota also (Morris & Blackwood, 2012). Synergism is similar to mutualism except the relationship is not required for the populations or organisms to live in the environment (Bottomley, 1998).

2.1.6. Summary of microbail function literature

Microorganisms are a key component of soil (Condron et al., 2010) as they play a vital role in maintaining organic matter, which influence overall soil health and fertility (Standing & Killham, 2012; Bardgett, 2016). Carbon gained from animal and plant detritus and plant root exudates is the main microbial energy source (Condron et al., 2010), and because of this, microbes are the main driver of carbon flux in the soil (Hopkins & Gregorich, 2005). However, some microbes can use N as their main energy source under specific conditions (Morris & Blackwood, 2012). Furthermore, microbes play a vital role in the N and P cycles, facilitating N mineralisation, nitrification, denitrification, and fixation of atmospheric N as well as mobilizing organic P (Hopkins & Gargait, 2010). In addition, fungi form a symbiotic relationship with plants in order to gain photo-assimilated carbon in return for improved P availability to the plants (Killham, 1994; Coleman et al., 2004; Allen, 1991). Nutrient availability is also impacted by both the positive and negative interactions between soil biota (Killham, 1994).

2.2. Assessing Soil Microbial Function

Soil microbial function is assessed and quantified using various methods based on physiological processes (Bloem, Hopkins, & Benedetti, 2005; Coleman, Crossley, & Hendrix, 2004); the most common methods being enzyme assays and microbial respiration (Tilston et al., 2010). Enzyme assays can be used to measure internal and external enzyme activity as well as the microbial demand for inorganic nutrients (Whalen & Sampedro, 2010). Microbial

respiration can be measured as either basal respiration, in the lab or in the field, or as substrate-induced respiration (SIR) (Pell et al., 2005). The Biolog and MicroResp™ systems are used to measure SIR using colour changing detection gels (Blagodatskaya & Kuzyakov, 2013). Another method of measuring microbial activity is the use of a microbial marker, such as adenosine triphosphate (ATP), which transports chemical energy within cells and is essential for living cells (Flieβbach & Widmer, 2005). The advantages and disadvantages of these methods arise from the simplicity and cost against the accuracy of the results to represent field conditions and actual microbial activity (Bloem, Hopkins, & Benedetti, 2005).

2.2.1. Enzyme Assays

Enzyme assays are the most commonly used method to measure microbial activity (Tilston et al., 2010). This measures the enzyme activities which are primarily attributed to bacteria and fungi as well as plant roots (Shaw & Burns, 2005). These assays can either be non-specific or specific (Tilston et al., 2010), however, specific assays relate only to specific reactions which may not reflect the actual level of organism activity (Coleman et al., 2004). The potential activity of enzymes of active microbial cells can indicate potential internal and external enzyme activity as well as the microbial demand for inorganic nutrients (Whalen & Sampedro, 2010). There are three principle methods which measure these processes; dehydrogenase activity, fluorescein diacetate hydrolysis, and *p*-nitrophenyl-linked substrate hydrolysis (Shaw & Burns, 2005). Dehydrogenase activity, using tetrazolium salts, indicates the actual respiratory activity of the soil microbial community (Shaw & Burns, 2005; Whalen & Sampedro, 2010). Fluorescein diacetate hydrolysis indicates the potential degradative activity by measuring extracellular enzymes involved in decomposition, such as lipase, protease, and esterase (Whalen & Sampedro, 2010; Shaw & Burns, 2005). *p*-nitrophenyl-linked substrate hydrolysis is a process which can be used to determine the activity of hydrolytic enzymes involved in specific processes such as phosphatases, sulfatases, glycosidases, and urease (Shaw & Burns, 2005). However, hydrolysis is not an entirely accurate measure of microbial activity as the extracellular enzymes may be associated with dead cells or complexed with clay and organic colloids (Shaw & Burns, 2005; Taylor et al., 2002). Therefore, dehydrogenase activity is expected to be more highly correlated with the actual level of soil microbial activity (Taylor et al., 2002).

Enzyme assays are significant in quantifying soil quality in terms of microbial activity due to the major contribution of microbial enzymes to the degradation of soil organic matter

(Trasar-Cepeda et al., 2000). However, the reliability of enzyme assays has been debated due to the misapplication of techniques and the misinterpretation of results (Coleman et al., 2004). Conditions used for the analysis, such as incubation and pH levels, determine the rate of catalysis (Taylor et al., 2002). These are manipulated to optimum levels for enzyme activity, standardising results so that they can be compared, but creating artificial conditions which are not encountered naturally (Shaw & Burns, 2005). Also of concern is the change in environmental parameters determining enzyme activity, which alters enzyme activity and results (Tate, 2002). This occurs primarily through the physical disruption of soil structure during sampling and preparation, which increases the accessibility of substrate and, therefore, microbial activity (Shaw & Burns, 2005). The interpretation of results gained by this process is also difficult (Taylor et al., 2002). There are underlying mechanisms which could impact results, such as the growth or death of microbes, the de-repression or repression of enzymes, and the inhibition or activation of enzymes (Shaw & Burns, 2005). However, the process is most commonly used since no expensive, sophisticated instruments are required to determine the results (Trasar-Cepeda et al., 2000).

2.2.2. Microbial Respiration

2.2.2.1. Basal Respiration

Microbial respiration is commonly used to measure microbial activity (Tilston et al., 2010) as it indicates the overall metabolic status of the microbial community (Coleman et al., 2004). The rate of microbial respiration is driven by substrate availability (Blagodatskaya & Kuzyakov, 2013), and is also influenced by soil water content and O_2 concentration (Pell et al., 2005). Therefore, where O_2 is not available, as in anaerobic conditions, the measurement of soil microbial activity is much more complex (Kandeler, 2007). Basal microbial respiration is determined by measuring either CO_2 release or O_2 uptake (Coleman et al., 2004; Kandeler, 2007) which represent carbon mineralisation and aerobic respiration respectively (Pell et al., 2005). Measurements can be undertaken in the field or in the laboratory (Whalen & Sampedro, 2010). Field measurements such as gas chambers or probes give reliable measurement of microbial activity in natural conditions (Kandeler, 2007), however, they include other organisms' respiration under highly variable special and temporal conditions (Pell et al., 2005). Laboratory techniques are more straightforward and use standardised conditions in order to compare results. However, due to the disturbance of sampling, sieving and storing the soil, results may not accurately reflect the actual activity in the field (Stotzky,

1965). The ratio of CO_2 output and O_2 uptake is known as the respiration quotient (RQ) and is useful for determining trends over time in a soil, or to compare similar soils, rather than basal respiration (Pell et al., 2005). The RQ can indicate the primary sources of carbon being metabolised in the soil (Coleman et al., 2004) and can be correlated directly with microbial activity (Stotzky, 1965). Basal respiration is a relatively insensitive method in monitoring soil health, however, addition of substrate can increase the sensitivity and accuracy of the method (Pell et al., 2005).

2.2.2.2. Substrate-Induced-Respiration

Substrate induced respiration (SIR) is used to measure the respiration of active glucose-responsive microorganisms in the soil and can be used to measure the total microbial biomass (Blagodatskaya & Kuzyakov, 2013; Anderson & Domsch, 1978). The method involves supplying glucose or another form of carbon to the soil, stimulating microbial activity, which is then measured by the resulting respiration compared with a control (Coleman et al., 2004; Whalen & Sampedro, 2010). This is based on the principle that when carbon is added in excess, under standardised water content and temperate conditions, the metabolism of carbon is only limited by the number of active microorganisms (Hóper, 2005). Respiration is followed for the first few hours in which time there is no increase in microbial population and therefore the response is proportional to the amount of microbial biomass in the soil (Kandeler, 2007; Hóper, 2005). The advantages of SIR include the objectivity and simplicity (Anderson & Domsch, 1978), the high speed and low cost (Kandeler, 2007), and accuracy (Hóper, 2005) of the procedure. However, deviations can occur in the results due to the age of microbial cells or faunal impacts (Anderson & Domsch, 1978), as well as the pH of the soil (Kandeler, 2007). It is considered to be a 'black box' method as there is no differentiation between fungal and bacterial respiration (Hóper, 2005). However, with the addition of certain inhibitors, the fungal to bacterial ratio can be measured (Kandeler, 2007), but this cannot be interpreted as actual fungal or bacterial biomass (Blagodatskaya & Kuzyakov, 2013).

2.2.2.2.1. Biolog

The Biolog system of measuring SIR is used to identify community-level physiological profiles (CLPP) and functional diversity of microorganisms based on utilisation of different carbon sources (Kandeler, 2007). It has proven effective in distinguishing spacial and temporal changes in the microbial communities analysed (Garland, 1997). Figure 5 shows how the

Biolog microplates are used for this process. However, the technique is thought to be inferior as it does not reflect microbial growth in the soil (Blagodatskaya & Kuzyakov, 2013; Degens & Harris, 1997). This is primarily due to the physical alteration by suspending soil extracts, but also because only a small proportion of the microbial population are able to proliferate under Biolog conditions (Rutgers et al., 2005). The major advantage of this process is the simplicity with low labour requirements (Garland, 1997). However, the acquisition and interpretation of the data needs careful attention (Rutgers et al., 2005).

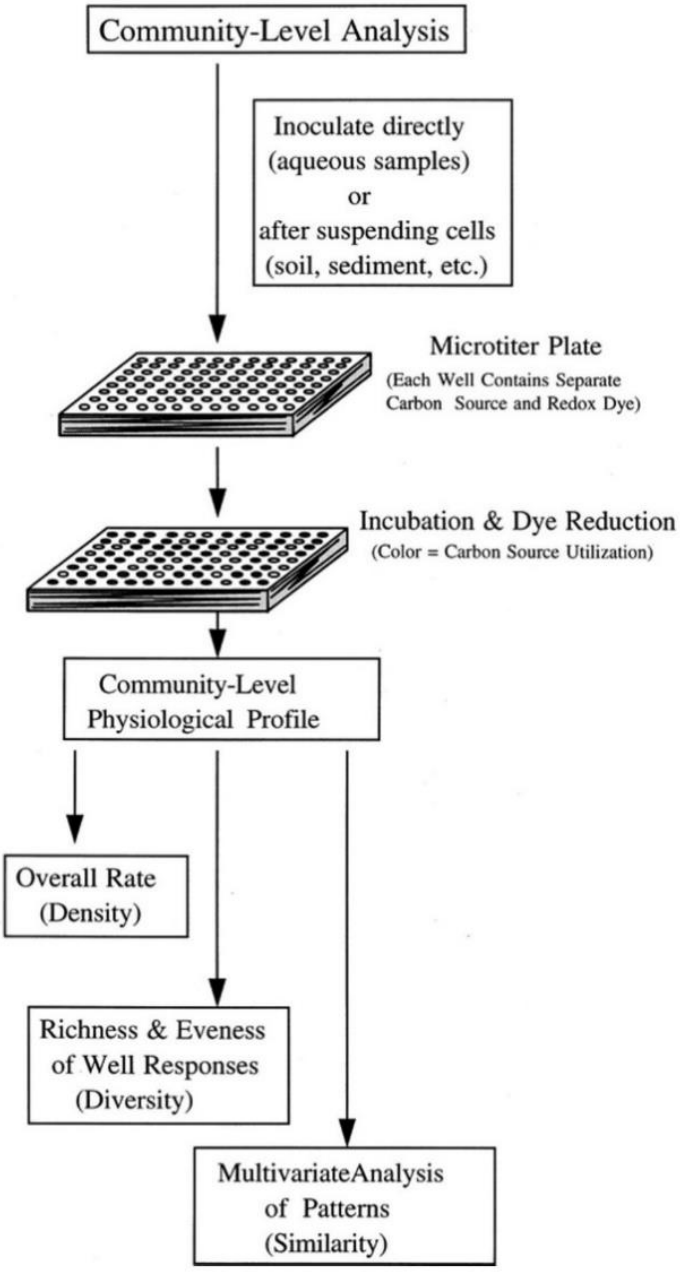

Figure 5. Community-level analytical approach for carbon source profiling using Biolog microplates (Adapted from Garland, 1997).

2.2.2.2.2. MicroResp™

MicroResp™ is a system which has been developed to measure SIR (Campbell et al., 2003; 2008) in order to directly measure the catabolic profile of the microbial community (Pignataro et al., 2012). The assembled MicroRespTM system is shown in Figure 6, where the two facing places are sealed together with a rubber gasket. Figure 7 shows the action of the individual well during the process. The detection plate changes colour at varying levels, depending on the level of CO_2 produced, which is measured (Campbell et al., 2003; 2008). Various carbon sources are added to individual wells (Campbell et al., 2003) along with a well with the addition of only water in order to test basal respiration (Campbell et al., 2008). This method allows the microbial population to be measured directly in the soil without extracting or culturing the organisms as with Biolog (Blagodatskaya & Kuzyakov, 2013; Degens & Harris, 1997), and has been reported to provide a greater level of discrimination (Banning et al., 2012).

Figure 6. A deep-well microtiter plate holds soil and a top plate holds a detection gel for MicroResp™. The connecting gasket seals the plates together (Retrieved from Campbell et al., 2003).

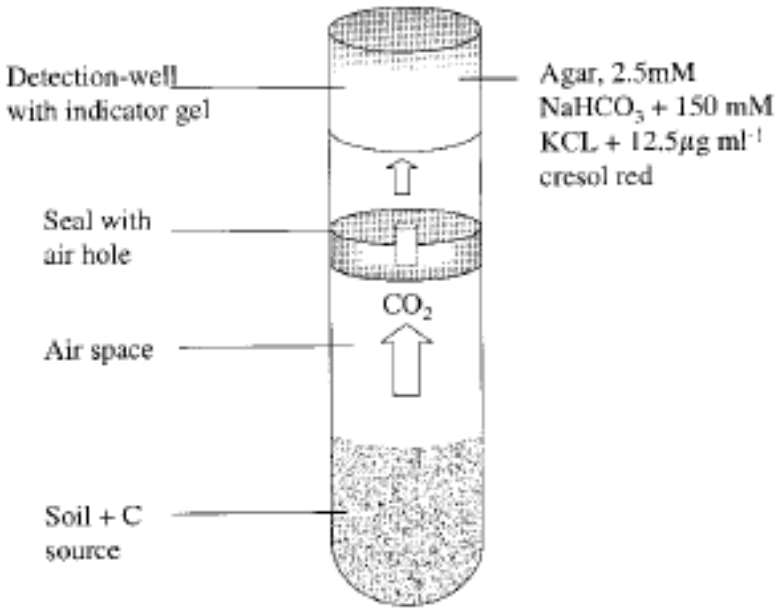

Figure 7. Schematic diagram of a deep well connected to a detection well, showing the position and composition of the dye detection system used in MicroResp™ (Retrieved from Campbell et al., 2003).

2.2.3. ATP Extraction

Adenosine 5'-triphosphate can be used as a molecular marker specific to living cells which is extracted as a measure of active microbial biomass (Flieβbach & Widmer, 2005). The amount of ATP extracted indicates total living organisms in the soil since all catabolic relations inside cells require ATP and it breaks down quickly in the soil (Kandeler, 2007). The method involves using either an acidic or alkaline extractant (Blagodatskaya & Kuzyakov, 2013) and produces stable results for ATP content of microbial biomass (Flieβbach & Widmer, 2005). However, the process includes the activation of dormant microbes and is therefore, not an accurate representation of microbes active naturally in the soil (Blagodatskaya & Kuzyakov, 2013).

2.2.4. Summary of microbial assessment literature

Soil microbial activity is assessed and quantified using various methods based on physiological processes (Bloem, Hopkins, & Benedetti, 2005; Coleman, Crossley, & Hendrix, 2004). Enzyme assays and microbial respiration are the most commonly used methods of measuring microbial activity (Tilston et al., 2010). These techniques use standardised conditions in the laboratory which may not accurately reflect the actual microbial activity in

the field (Shaw & Burns, 2005; Pell et al., 2005). However, respiration under field conditions also includes the effect of soil fauna and plant roots, and can be highly variable (Pell et al., 2005). Substrate-induced respiration is thought to be more sensitive to changes in the soil compared with basal respiration (Pell et al., 2005). This is measured using either the Biolog system or the MicroResp™ system. MicroResp™ more accurately represents field conditions as the Biolog system requires the soil to be suspended in solution (Degens & Harris, 1997). ATP extraction can be used as an index of microbial activity, however the activation of dormant microorganisms and the variation in techniques causes variation in published results (Blagodatskaya & Kuzyakov, 2013).

2.3. Research Objectives

The objectives of this study were to determine if long-term fertiliser application, long-term biomass removal and/or short-term dairy shed effluent application had a significant impact on microbial function in soil. Furthermore, the effect of season on soil microbial function was determined by taking samples in autumn, winter and spring. Samples of specific treatments were taken from two unique long-term trials; the Winchmore fertiliser trial and the Long-term Ecology Trial. In addition, a short-term trial of dairy shed effluent (DSE) application. Quantification of soil microbial function through MicroResp^{TM} was used to determine whether the method can be used effectively.

3. Materials and Methods

3.1. Field Trials

3.1.1. Long-Term Winchmore Fertiliser Trial

The Winchmore fertiliser trial is located at the Winchmore Irrigation Research Station in mid-Canterbury, New Zealand (171°48'E, 43°47'S), and was set up in 1952 as described in Condron et al. (2012). Briefly, the trial is situated on a Lismore stony silt loam soil, and has 0.9 ha plots which are flood irrigated (c. 1150 mm y^{-1}), with fences along boarders in order to prevent nutrient transfer between plots during irrigation events. There are four replicates of the five treatments arranged in randomised blocks with five separate flocks of sheep grazing each treatment to avoid nutrient transfer between treatments. The five treatments are a control (nil P), three rates of super phosphate application (188 kg SPP ha^{-1} y^{-1}, 250 kg SPP ha^{-1} y^{-1}, and 375 kg SPP ha^{-1} y^{-1}) and reactive phosphate rock application (175 kg Sechura $ha^{-1}y^{-1}$), as shown in Figure 8. The two treatments selected for this study were the control (nil P), and 376 kg SSP ha^{-1} y^{-1} (376PA).

Figure 8. Winchmore fertiliser trial treatment set up.

3.1.2. Long-Term Ecology Trial

The Long-Term Ecology Trial (LTET) is located at Lincoln University, New Zealand (S 43°38'51, E 172°28'05), and was established in 1994 as described in Simpson et al. (2012). Briefly, the trial is located on a Wakanui silt loam soil, and has 5 x 5 m plots sown with red clover (*Trifolium pratense* L. cv. Pawera), white clover (*Trifolium repens* L. cv. Tahora), perennial ryegrass (*Lolium Perenne* L.) and cocksfoot (*Dactylis glomerata* L. cv. Kahu). The trial has randomised blocks with four replicates of eight treatments, which are listed in Table 1. Mowed biomass is used interchangeably with clippings (C_R or C_L). The two treatments selected for this study were both mown irregularly and received no N-fertiliser with either clippings left ($M_IC_LN_0$), or clippings removed ($M_IC_RN_0$). Figure 9 is an image of the trial during the time of sampling.

Table 1. Description of treatments applied to the Long-Term Ecology Trial (Retrieved from Adiar, Wratten, & Lear, 2013)

Treatments	Mowing	Mowed Biomass	N-fertilizer
M_0N_0	None	–	None
M_0N_1	None	–	Added
$M_IB_RN_0$	Irregularly	Removed	None
$M_IB_RN_1$	Irregularly	Removed	Added
$M_IB_LN_0$	Irregularly	Left	None
$M_IB_LN_1$	Irregularly	Left	Added
$M_RB_LN_0$	Regularly	Left	None
$M_RB_LN_1$	Regularly	Left	Added

Figure 9. The Long-Term Ecology Trial at Lincoln University

3.1.3. Short-term Dairy Shed Effluent Trial

The short-term dairy shed effluent trial (DSE) trial was established adjacent to the LTET in August 2016 on pasture which has received the same management as the LTET, as described in Simpson et al. (2012). Dairy shed effluent treatments were applied, with four replicates, at rates of 50 (low) and 100 kg N ha^{-1} (high) on circular plots (diameter: 15 cm), along with water treatments of equal volumes (low; 360 mL, high; 720mL). The treatments were set out in a randomised complete block design, shown in Figure 10. Treatments were applied twice during August with three weeks between applications.

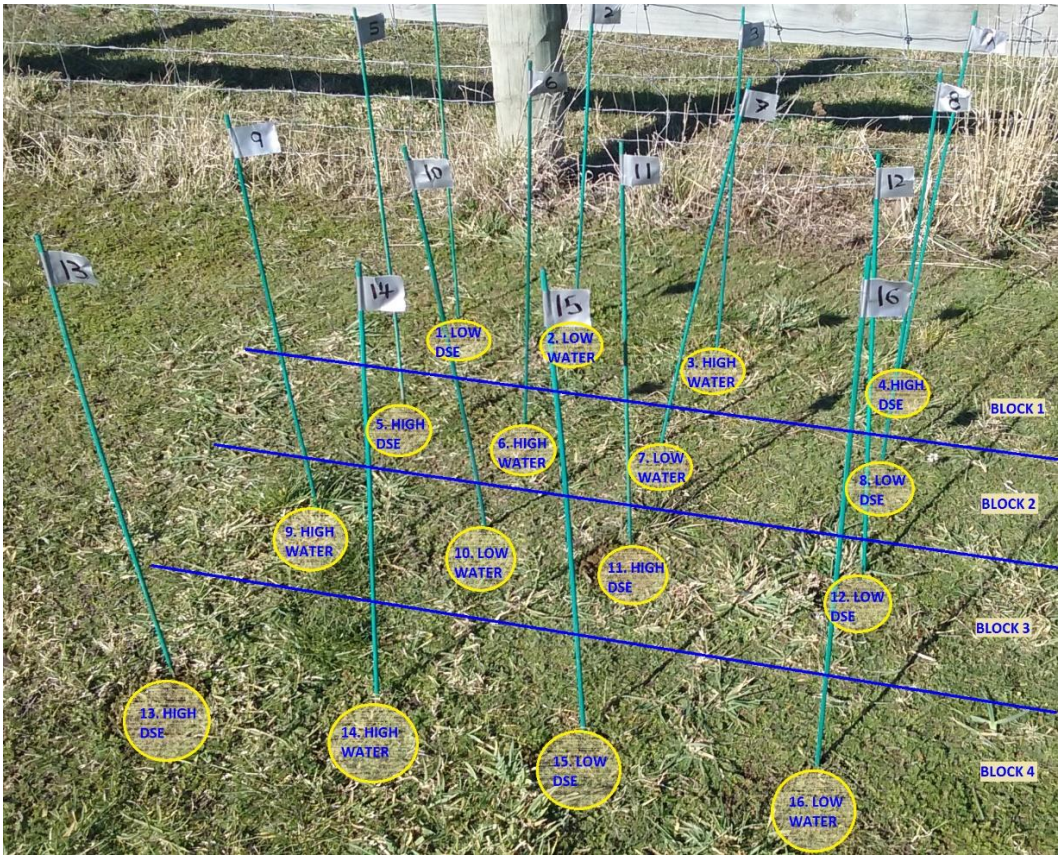

Figure 10. DSE short term field trial treatment allocation and blocking.

3.2. Soil sampling and analysis

Soil cores (0-7.5 cm) were taken from each plot of treatments stated above during autumn (Winchmore; March, LTET; May), winter (Winchmore and LTET; July), and early spring (Winchmore & LTET; September). The DSE trial sampling occurred three weeks after 'winter' application (August) and 4 weeks after 'early spring' application (September). All samples were sieved to <2 mm and analysed for basal and substrate-induced respiration using MicroResp™ as described by Campbell et al. (2003). Water was used as a control for basal

respiration along with 22 carbon sources; L-arabinose, D-fructose, D-galactose, a -D-glucose, D -Xylose, maltose, sucrose, raffinose, citric acid, glycoloc acid, tartaric, glycerol 50%, D-(+)-glucosamine, hydrochloride, Urea, triton x-100, L-proline, glycine, L-alanine, L-serine, arginine, cysteine, and tryrosine.

3.3. Statistical analysis and data

All measurements are reported as mean values with standard error. Statistical analysis for the data was carried out using 'RStudio' (version 3.2.3, RStudio, Inc. Boston, USA) as a platform for the statistical package R version 3.3.1 (R Core Team 2016), with $P = 0.05$ as the threshold for significance. Values for each carbon source were subjected to multivariate analysis of variance and least square means to determine significant differences between treatments and season. MicroResp^{TM} results are presented as respiration means from assays of experimental replicates with error bars indicating standard error of the mean. Results of respiration shown are those which are significantly different between seasons or treatment, indicated by different letters.

4. Results

4.1. Winchmore Fertiliser Trial

Results from Winchmore samples show that long-term fertiliser application did not have a significant effect on microbial function, except for two substrates added (Figure 11). Therefore, although the control treatment SIRs were significantly higher with triton x-100 application (P = 0.004) and significantly lower for arginine application (P = 0.025), these are not consistent compared with the rest of the results. There was no significant effect (P = 0.613) on basal respiration between control and superphosphate treatments.

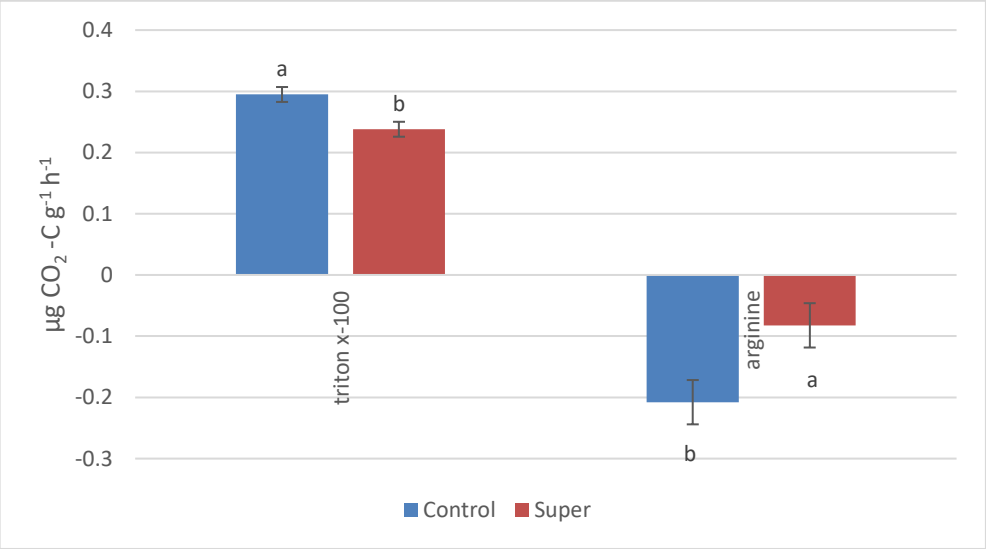

Figure 11. The effects of long-term fertiliser application on microbial function on the Long-Term Winchmore Fertiliser Trial measured using MicroResp™

Figure 12 shows that the season in which samples were taken had a significant effect on respiration. Basal respiration ranged from 0.06 to 0.16 µg CO_2 -C g^{-1} h^{-1}, where autumn was significant different (P = 0.030), however there was no significant difference between winter and spring. The autumn test tended to have the greatest SIRs as shown in Figure 12, except for arginine. For the most part, winter and spring SIRs are not significantly different, however, some substrates caused spring to be significantly lower than winter (L-arabinose, D-galactose, and urea), and some substrates cause spring to be significantly higher than winter (citric acid, glycolic acid, D- (+)-glucosamine hydrochloride, L-proline, arginine, cysteine, and tyrosine). In two cases spring SIRs are statistically similar to autumn SIRs (D-(+)-glucosamine hydrochloride, and cysteine).

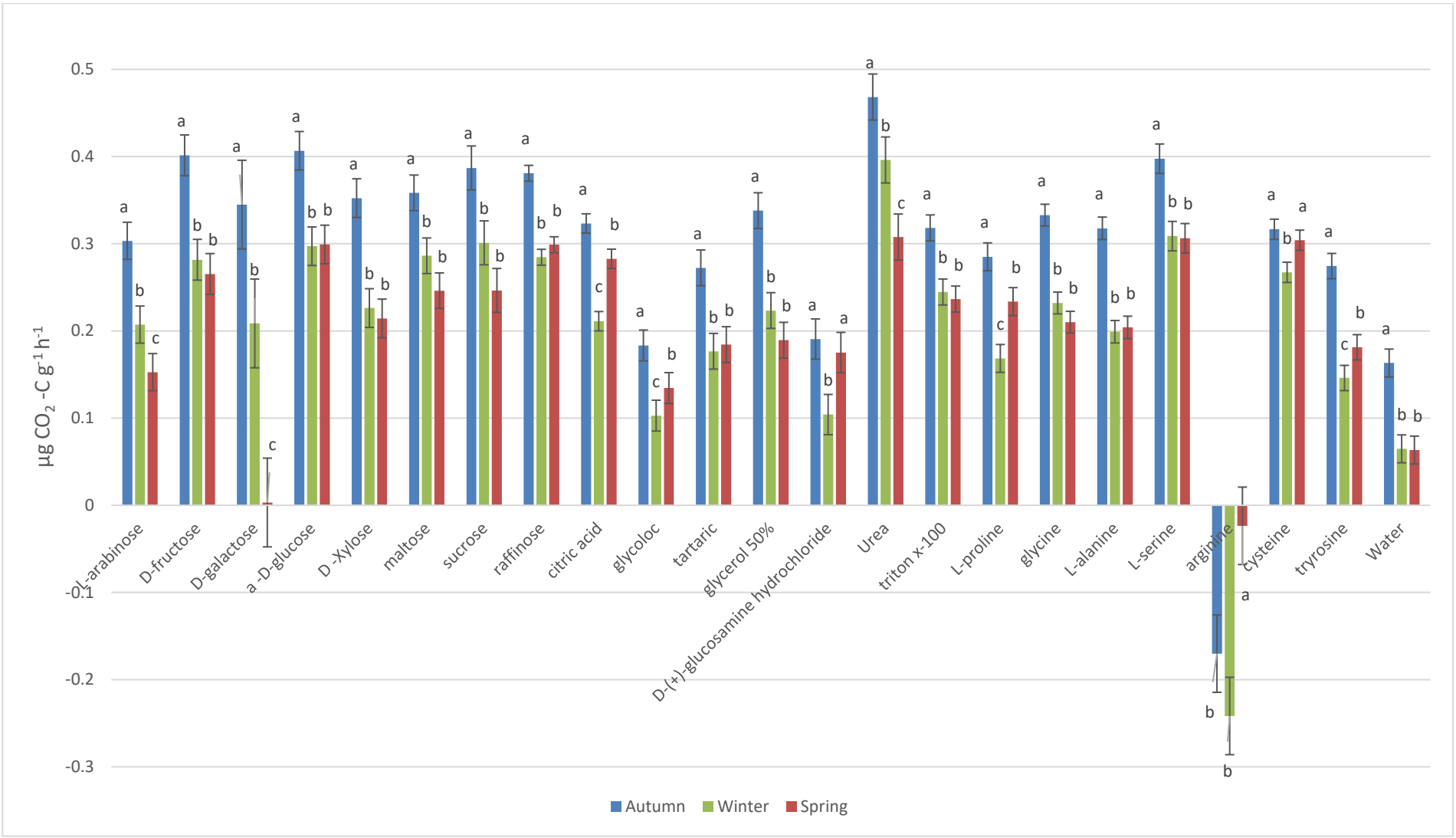

Figure 12. The effect of season on microbial function on the Long-Term Winchmore Fertiliser Trial measured using MicroResp™

4.2. LTET

Respiration was significantly different between treatments of clipping left and clippings removed on the LTET (Figure 13). The significant SIRs ($P < 0.050$) obtained show the microbial respiration is significantly higher under treatments where nutrients are returned to the soil through clippings compared with removing nutrients. However, there was no significant difference found between treatment basal respiration with means of 0.11 and 0.14 µg CO_2 -C g^{-1} h^{-1} for clippings removed and clippings retained respectively ($P = 0.233$).

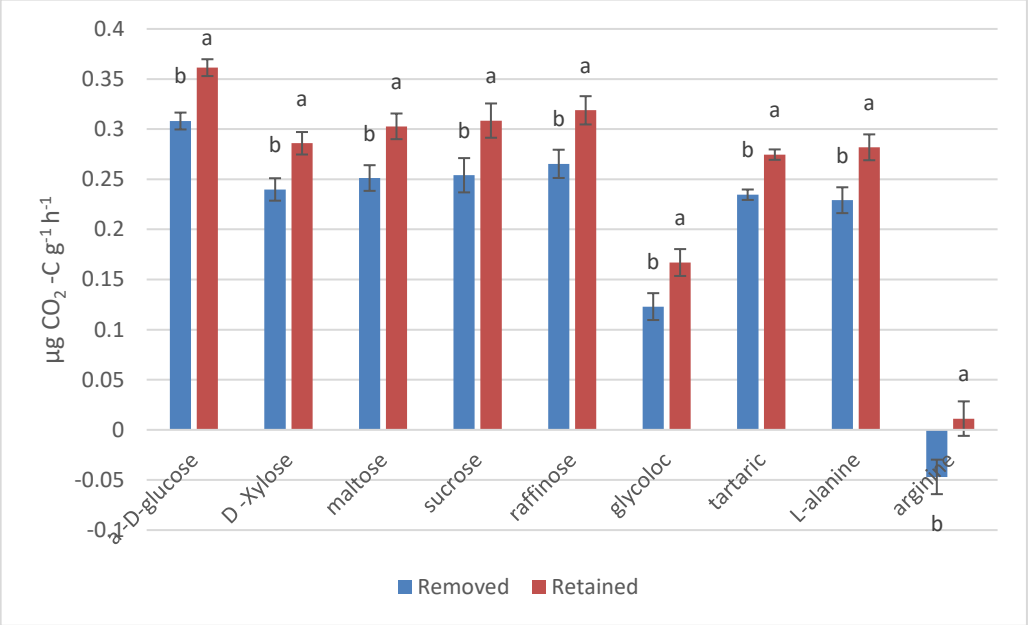

Figure 13. The effect of long term biomass removal and retention on microbial function on the LTET measured using MicroResp™.

Season had a significant effect on microbial function measured by MircoResp™ on the LTET (Figure 14). This is shown primarily by the measure of basal respiration which ranged from 0.04 to 0.19 µg CO_2 -C g^{-1} h^{-1} where, autumn > spring > winter ($P = 0.020$). However, only one significant SIR result showed the same trend (tartaric). The most common significant SIRs followed the trend of autumn > winter = spring (L-arabinose, D-fructose, maltose, glycoloc, glycerol 50%, D- (+)-glucosamine hydrochloride, triton x-100, and L-serine), followed by the trend autumn > winter > spring (D-galactose, a -D-glucose, D -Xylose, sucrose, and raffinose). For three SIRs, the trend was autumn = spring > winter (glycine, L-alanine, and tyrosine), however, for two cases spring SIRs exceeded autumn (citric acid, and L-proline). Urea and arginine resulted in different SIR trends altogether, of autumn = winter > spring, and spring > autumn = winter respectively.

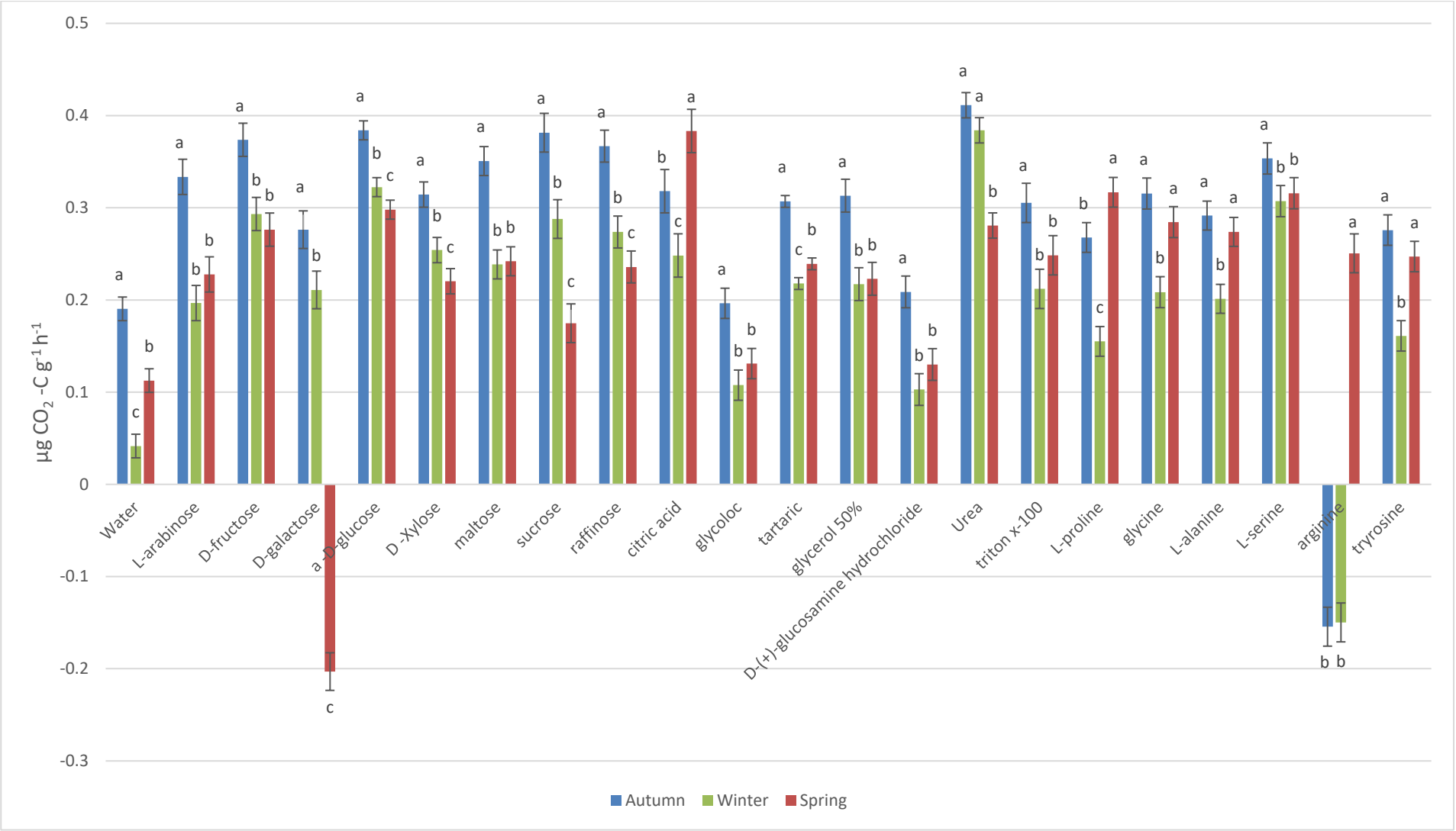

Figure 14. The effect of season on microbial function on the LTET measured using MicroResp™.

4.3. DSE Trial

Microbial respiration measurement using MircoResp™ resulted in one significant difference between short term DSE and water application ($P = 0.006$) (Figure 15). As the majority of the substrates were not significantly different ($P > 0.050$), glycerol 50% was considered abnormal, therefore, no significant difference was found between applying DSE and water. Basal respiration rates were not significantly different for DSE and water treatments at 0.13 and 0.15 µg CO_2 -C g^{-1} h^{-1} respectively.

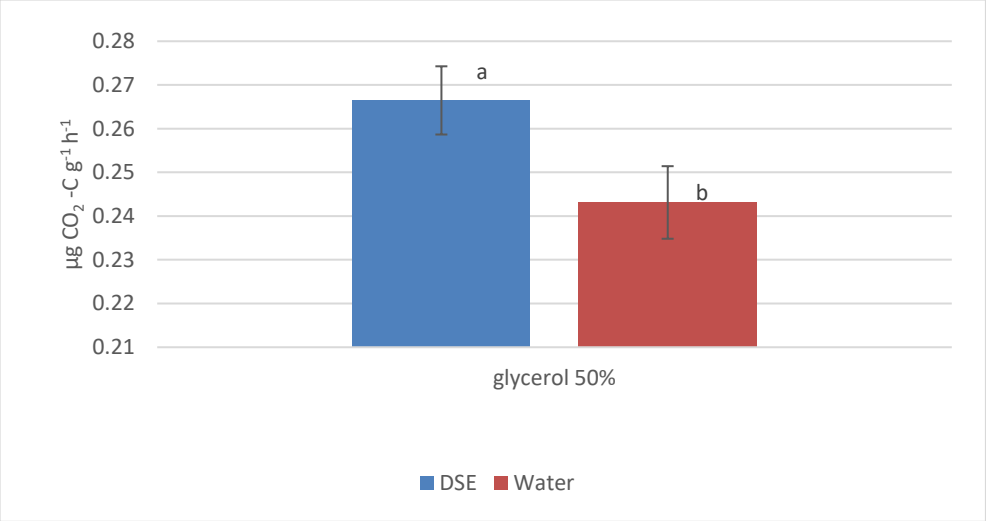

Figure 15. The short-term effects of dairy shed effluent application on microbial function measured by MicroResp™.

MicroResp™ results indicate seasonality effected microbial function on the DSE short-term trial (Figure 16). Basal respiration ranged from 0.06 to 0.18 µg CO_2 -C g^{-1} h^{-1} with spring values significantly greater than winter values ($P = 0.017$). This trend is also shown by the significant SIRs ($P < 0.050$), for which all but one substrate (D-galactose) induced higher spring microbial respiration.

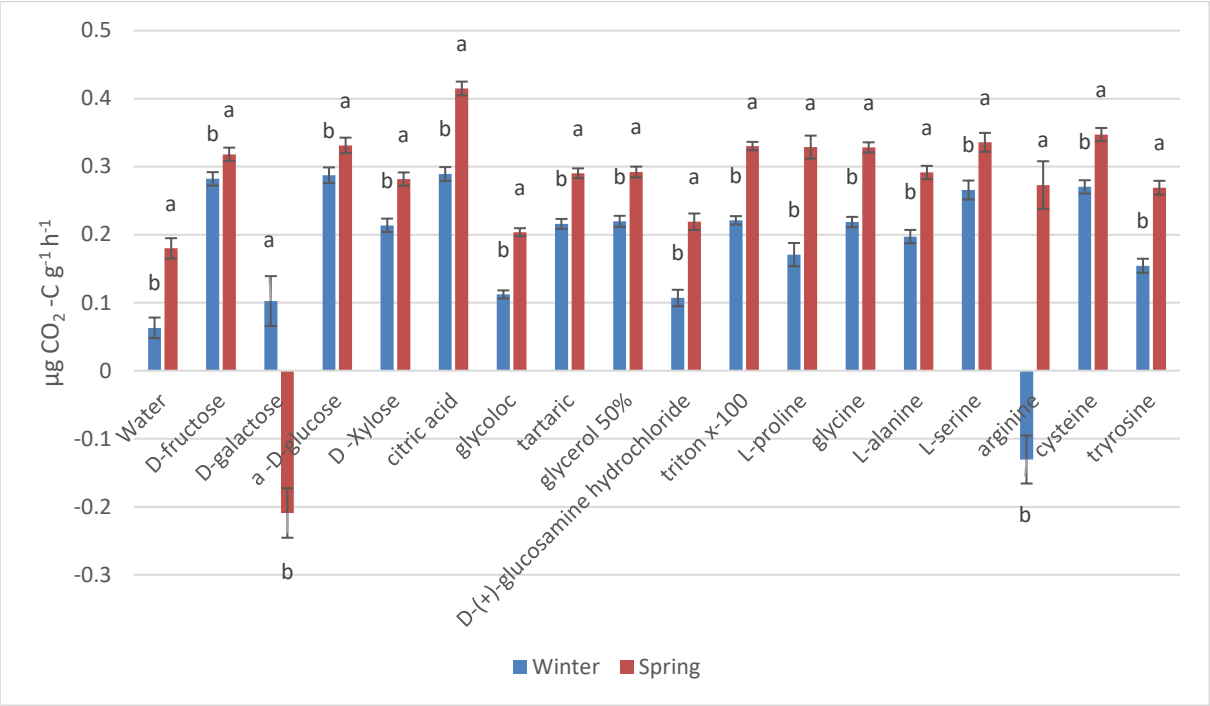

Figure 16. The effect of season on microbial function on the DSE trial measured by MicroResp™.

5. Discussion

Interpreting MicroResp^{TM} data can be complicated due to the quantity and range of data collected for each soil sample when amended with water and range of soluble carbon and carbon-nitrogen compounds. While addition of water Indicates basal respiration, the catabolic response to the addition of the various carbon and carbon-nitrogen substrates (substrate induced respiration) can provide information on the functional ecology of the soil microbial population (Campbell et al., 2003). In particular, the quantity of added substrate utilised by the soil microbial biomass may reflect differences in microbial diversity, although this can vary for different substrates (e.g. L-arginine) (Creamer et al., 2009; 2016).

Results for the long-term fertiliser trial at Winchmore unexpectedly suggest that the addition of fertiliser for over 60 years and its consequent effect on markedly increasing pasture production (Smith et al., 2012) had no significant impact on basal respiration (0.09 μg CO_2 -C g^{-1} h^{-1}) or substrate induced respiration for the range of compounds added. Microbial activity has been shown to be most sensitive to levels of organic carbon (organic matter) and acidity in soil (Bünemann et al., 2006). Condron et al. (2012) found that after 57 years there were no significant differences in either soil organic carbon or pH between the nil P and 376PA fertiliser treatments at Winchmore. This in turn may at least partly explain the absence of any difference in basal and substrate induced respiration between the nil P and 376PA treatments observed in the present study. However, it is possible that there were in fact differences in the diversity of the microbial communities in these soils, and other studies have found differences in specific groups of microorganisms in response to P fertiliser at Winchmore (Wakelin et al., 2012).

As expected, basal respiration was higher in autumn compared with mid-winter and early spring at Winchmore, which was also reflected in the response to the addition of a wide range of carbon and carbon-nitrogen substrates. This seasonal pattern can be mainly attributed to the impact of soil temperature on microbial activity, given that topsoil temperatures in Canterbury are lowest in mid-winter (~5 ^{o}C) compared with autumn and early spring (7-10 ^{o}C) (MetService, 2016). Soil moisture was unlikely to be factor influencing soil microbial activity at Winchmore over the sampling period since these soils receive adequate rainfall between April and October (soil moisture: May: 23%, mid-winter: 33%,

early spring: 30%), and irrigation over the remaining months of the year. These observations are consistent with data from other studies which have clearly demonstrated that soil microbial activity is influenced by a combination of soil temperature and moisture (Arnold et al., 1999; Blume et al., 2002; Tilston et al., 2010)

Soil microbial respiration data for the LTET at Lincoln University over the same sampling period (i.e. autumn, mid-winter, early spring) were very similar to data from Winchmore over the same period. Thus, soil microbial respiration response to the addition of a wide variety of substrates was significantly higher in autumn compared with mid-winter and spring, which again could be attributed to the effect of temperature on soil microbial activity. However, respiration for several substrates was higher in early spring compared with mid-winter at LTET and not at Winchmore. This may reflect higher soil temperatures in early spring at Lincoln compared with Winchmore, since the location of Lincoln is relatively closer to the coast.

In contrast to results from Winchmore, data from LTET clearly demonstrated that microbial response to the addition of selected carbon substrates was consistently and significantly higher for the biomass retained compared with the biomass removed treatments. These substrates comprised 5 sugars (glucose, xylose, maltose, sucrose, and raffinose), together with 2 low molecular weight organic acids (glycolic and tartaric) and a single amino acid (alanine). The fact that most of these were mainly carbon-only substrates tentatively indicates that differences between the microbial populations in the contrasting treatments was related to differences in the energy demands rather than demand for nitrogen. The enhanced respiration observed for the biomass retained treatment compared with biomass removed may be mainly attributed to the fact that soil organic carbon was significantly higher for the biomass retained (3.9%) than biomass removed (3.3%), while soil pH was similar for both treatments (5.6-5.8) (Simpson et al., 2012). This is also consistent with the absence of a treatment effect at Winchmore as discussed above. The apparent difference in substrate respiration observed between the biomass retained and removed treatments on the LTET may also partly reflect changes in the composition and diversity of the microbial community. Adair et al. (2013) observed significant differences in the diversity of soil bacteria under biomass retention compared with removal, which they attributed to a combination of changes in soil chemistry (including P availability) and plant community,

although they did not consider the possible influence of differences in soil organic carbon on bacterial diversity.

There were significant seasonal differences observed in basal and substrate induced respiration for the short-term DSE trial whereby respiration was higher in early spring compared with mid-winter, which was consistent with data from the long-term Winchmore and LTET trials as discussed above. However, apart from a single carbon substrate (glycerol), there were no significant differences in respiration response observed between the addition of water and DSE at two rates. Dairy shed effluent at the application rates employed contains soluble and particulate organic carbon together with N (355-490 mg/l), P (70 mg/l), potassium (370 mg/l), and S (25-155 mg/l) (Longhurst et al., 2000). Accordingly, it was expected that the addition of DSE would elicit some change in soluble substrate addition response by soil microbes compared with water. However, the results suggest that the concentrations of organic carbon and nutrients in DSE were not sufficient to cause a significant increase in soil microbial community activity or composition.

Consistent with findings form the adjacent LTET long-term trial, the immediate impact of adding DSE on basal respiration was higher in spring (0.17 µg CO_2 -C g^{-1} h^{-1}) than winter (0.11 µg CO_2 -C g^{-1} h^{-1}). This may be partly attributed to the increase in temperature between sampling times, however, as the samples were only taken a month apart, with the 'spring' sample taken in very early spring/late winter, temperature is most like not the main factor, but rather soil moisture. There was a significant increase in soil moisture between sampling as DSE or water was reapplied after the first sample was taken. Both factors may have influenced microbial activity and biomass (Blume et al., 2002).

6. Conclusions

The findings of this study clearly demonstrated that environmental conditions in the field, principally soil temperature, and to a lesser degree soil moisture, were the primary factors that influenced soil microbial activity and function. This was determined by the catabolic response to the addition of water and a range of carbon and carbon/nitrogen compounds under controlled conditions. Unexpectedly, the long-term addition of fertiliser phosphorus and sulphur (with consequent enhanced biological fixation of nitrogen) at Winchmore did not have any significant impact on soil microbial function, which was mainly attributed to the fact that organic carbon and pH were similar in the unfertilised and fertilised soils. On the other hand, long-term retention or removal of biomass under ungrazed grassland did have a significant impact on soil microbial function which reflected differences in soil organic carbon under the contrasting management regimes.

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Appendix A

MicroResp™ Mean Results

Mean MicroRespTM Results ($\mu g\ CO_2$ -C $g^{-1}\ h^{-1}$)	Long-term Winchmore fertiliser trial						Long-Term Ecology trial						Short-term DSE trial			
	Autumn		Winter		Spring		Autumn		Winter		Spring		Winter		Spring	
	nil P	376 PA	nil P	377 PA	nil P	378 PA	C REM	C RET	C REM	C RET	C REM	C RET	DSE	Water	DSE	Water
Water	0.12	0.14	0.10	0.04	0.08	0.07	0.18	0.20	0.05	0.03	0.08	0.15	0.06	0.07	0.16	0.20
L-arabinose	0.32	0.29	0.21	0.21	0.19	0.12	0.33	0.33	0.19	0.20	0.18	0.27	0.19	0.18	0.20	0.22
D-fructose	0.40	0.40	0.25	0.31	0.28	0.25	0.34	0.41	0.30	0.29	0.28	0.27	0.30	0.26	0.31	0.33
D-galactose	0.35	0.34	0.21	0.21	0.12	-0.11	0.27	0.28	0.18	0.24	-0.20	-0.21	0.12	0.08	-0.21	-0.21
a -D-glucose	0.41	0.40	0.28	0.31	0.32	0.28	0.35	0.42	0.31	0.33	0.26	0.33	0.30	0.27	0.32	0.35
D -Xylose	0.36	0.34	0.20	0.25	0.24	0.19	0.30	0.33	0.23	0.28	0.19	0.25	0.23	0.20	0.27	0.29
maltose	0.35	0.36	0.30	0.27	0.27	0.23	0.33	0.37	0.21	0.27	0.21	0.27	0.26	0.23	0.26	0.28
sucrose	0.39	0.39	0.29	0.31	0.28	0.22	0.35	0.41	0.27	0.30	0.13	0.21	0.24	0.19	0.24	0.28
raffinose	0.37	0.40	0.28	0.28	0.29	0.30	0.33	0.40	0.27	0.28	0.20	0.27	0.25	0.24	0.27	0.29
citric acid	0.32	0.32	0.21	0.21	0.25	0.32	0.29	0.35	0.26	0.24	0.36	0.40	0.29	0.29	0.41	0.42
glycoloc	0.18	0.19	0.08	0.12	0.10	0.17	0.18	0.22	0.10	0.11	0.09	0.17	0.12	0.11	0.20	0.21
tartaric	0.29	0.25	0.17	0.18	0.17	0.20	0.29	0.33	0.21	0.22	0.20	0.27	0.22	0.21	0.29	0.29
glycerol 50%	0.33	0.35	0.22	0.23	0.19	0.19	0.32	0.30	0.23	0.21	0.19	0.25	0.23	0.20	0.30	0.29
D-(+)-glucosamine hydrochloride	0.20	0.18	0.11	0.10	0.21	0.14	0.20	0.22	0.09	0.11	0.09	0.17	0.11	0.10	0.24	0.20
Urea	0.47	0.46	0.40	0.39	0.37	0.25	0.40	0.42	0.38	0.38	0.25	0.31	0.34	0.31	0.34	0.31
triton x-100	0.34	0.30	0.25	0.24	0.29	0.18	0.30	0.31	0.20	0.22	0.22	0.28	0.24	0.20	0.32	0.34
L-proline	0.27	0.30	0.16	0.18	0.21	0.26	0.27	0.26	0.15	0.16	0.28	0.35	0.17	0.18	0.33	0.32
glycine	0.33	0.33	0.24	0.22	0.24	0.18	0.30	0.33	0.24	0.18	0.26	0.31	0.22	0.21	0.32	0.33
L-alanine	0.32	0.32	0.19	0.20	0.19	0.22	0.26	0.32	0.20	0.20	0.23	0.32	0.21	0.18	0.29	0.29
L-serine	0.38	0.42	0.31	0.31	0.29	0.32	0.33	0.38	0.28	0.33	0.29	0.34	0.28	0.24	0.35	0.32
arginine	-0.20	-0.14	-0.25	-0.23	-0.18	0.13	-0.17	-0.14	-0.18	-0.12	0.20	0.30	-0.14	-0.12	0.28	0.26
cysteine	0.34	0.30	0.28	0.26	0.28	0.33	0.22	0.31	0.18	0.23	0.26	0.35	0.28	0.26	0.35	0.35
tyrosine	0.26	0.28	0.14	0.15	0.19	0.18	0.26	0.29	0.16	0.17	0.21	0.28	0.17	0.13	0.27	0.26
Water	0.16	0.17	0.06	0.07	0.06	0.07	0.17	0.20	0.09	0.09	0.10	0.16	0.17	0.16	0.15	0.18